An examination of the effects of chronic static and fluctuating temperature on the growth and survival of spikedace, *Meda fulgida*, with implications for management

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Abstract

Stream temperatures are on the rise in small streams in southern Arizona. Although, the biology of the spikedace, *Meda fulgida*, has been relatively well studied, little information is known about the thermal tolerance of this species and how rising temperatures impact its survival. We used the chronic lethal method and the acclimated chronic exposure (ACE) method, for both static and fluctuating temperatures, to estimate the thermal tolerance of spikedace. All three methods utilize a slow acclimation period, which mimics the natural environment more successfully than traditional thermal methods. These methods also allow for the evaluation of sub-lethal effects of temperature such as slowed growth and susceptibility to disease. The ACE method using static temperatures provided the most conservative estimate of thermal tolerance. The estimated 30 d LT50 was 32.1°C. Fish exposed to the 32°C treatment experienced only 37% of the growth experienced by control fish. Multiple behavioral and physiological changes, indicative of stress, were noted at 30, 32 and 33°C treatments. A modified ACE method allowed us to incorporate temperature fluctuations ranging from 4 to 10°C. We observed 96.5% survival for fish exposed to the control (25°C) treatment. Similar survival rates were observed for after 30 d exposure to a 24–34°C fluctuation. We saw a significant decrease in survival for fish exposed to the 28–34°C fluctuation (mean survival = 79.2%) and 30–34°C fluctuation (mean survival = 73.3%). Fish held at both the 24–34°C and 30–34°C fluctuations showed significantly lesser growth than the control treatment. Under natural conditions, tolerance may be affected by synergistic interactions with other environmental stressors such as predation and poor water quality. Determination of thermal tolerance for native fishes is critical to estimate available habitat and may provide insight into reasons for their decline.

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1. Introduction

Temperature influences nearly all biochemical, physiological, and life history activities of a fish (Beitinger et al., 2000; Poole and Berman, 2001) and maintaining a specific range of temperatures is critical for the conservation of freshwater fishes. Water temperature is one of many factors that can directly influence the distribution of fishes in a stream (Brett, 1956; Castleberry and Cech, 1986; Myrick and Cech, 2000; Lass and Spaak, 2003) and alteration of natural water temperature regimes can cause a wide variety of behavioral and physiological responses with the most dramatic being death (Fry, 1967; Lutterschnidt and Hutchison, 1997). Furthermore, temperature can influence metabolic activities, having protracted effects on behaviors such as spawning and predator avoidance. High temperatures can indirectly influence survival by promoting unfavorable conditions such as crowding in small pools where temperature gradients are more favorable (Deacon and Minckley, 1974), therefore increasing exposure to disease or predation.
Within the past 100 years many controlled laboratory methods have evolved, incorporating both static and dynamic methodologies (Huntsman and Sparks, 1924; Cowles and Bogert, 1944; Brett, 1956; Becker and Genoway, 1979) and as a result thermal tolerance data exists for most North American species. Despite this thermal tolerance data is still lacking for many native fishes in the Southwest even though increasing stream temperatures may be contributing to their decline. Small streams in the Southwest can experience extreme temperature fluctuations (Moyle and Vondracek, 1985). Areas with shallow stagnant water, high ambient temperatures, and direct exposure to solar radiation are common in small southwestern streams, and are particularly vulnerable to rising temperatures. Temperatures in relatively undisturbed systems in Arizona can fluctuate up to 15–20 °C d⁻¹ (Deacon and Minckley, 1974; US Geological Survey, 2006). These fluctuations are further exacerbated in systems where historical hydrological patterns have been altered. Straighter, wider stream beds combined with the destruction of riparian vegetation and decreased stream flow expose more water surface to solar radiation and increase convective heat gain (Dickerson and Vinyard, 1999). Natural mortality in fishes related to high temperatures has been recorded frequently in small southwestern streams (Deacon and Minckley, 1974).

Increasing water temperatures are prevalent in habitat historically utilized by the spikedace, *Meda fulgida*, currently listed as threatened under the US Endangered Species Act. Although the biology of this small stream dwelling cyprinid has been well studied (Barber et al., 1970; Barber and Minckley, 1983; Probst et al., 1986; Rinne, 1991; Douglas et al., 1994; Ward et al., 2003) little information is available on the thermal tolerance of this species. The objective of our study was to estimate the upper thermal tolerance of the spikedace using three well-tested methods that incorporate both chronic and fluctuating treatments. A determination of upper thermal tolerance limits for the spikedace will assist in defining habitat requirements and developing appropriate strategies for species recovery.

2. Methods

2.1. Fish collection

We used 4-m long seine nets (mesh size 1.6 mm) to collect spikedace in spring and summer 2003 and 2004 from Aravaipa Creek, Arizona. Due to concern over the Aravaipa Creek spikedace population, we used captive bred fish from Aravaipa Creek and mixed them randomly with wild fish. The University of New Mexico and Bubbling Ponds State Fish Hatchery, Arizona provided age-0 fish. We treated wild fish for Ichthyophthirius, using formaldehyde (Quickcure®), for at least 5 d. Approximately 20% of the spikedace collected from Aravaipa Creek were infected with yellow grub, Clinostomum complanatum. These fish were excluded from this experiment.

We fed all fish daily to satiation with a combination of brine shrimp, daphnia, bloodworms, spirulina, and tropical fish food flakes. Unconsumed food was removed every other day by siphon. Fish were not fed 24 h prior to testing.

2.2. Acclimated chronic exposure (ACE) method with static temperatures

We conducted temperature tolerance tests using the acclimate chronic exposure (ACE) method (Zale, 1984; Selong et al., 2001). The ACE method is a hybrid of two traditional methods, the critical thermal methodology (CTM) and the incipient lethal temperature (ILT). The ACE method allows full acclimation to changing temperatures by gradually increasing temperatures until a test temperature is reached. A full review of the ACE methodology is available in Selong et al., 2001.

Experiments were conducted from October 2003 to December 2003. Fish were measured to the nearest millimeter, after a 24 h fasting period, to document the effects of temperature on growth and to select fish of similar size (20–40 mm). Spikedace averaged 31.6 mm ± 0.5 (TL) and 0.21 g ± 0.01 (95% CI). We measured all fish and randomly assigned 10 fish to each test tank. Each 72 L aluminum tank (122 cm × 36 cm × 25 cm deep) was equipped with a sponge filter, 10 cm airstone, powerhead (Rio 1100), and 200 W Ebo-Jager aquarium heater. The powerhead simulated natural stream currents and prevented hot spots in tanks. Tanks were covered with foamboard lids with small windows to allow light penetration. Timers maintained light cycles in the building at an Arizona summer photoperiod (14 h light:10 h dark). Heaters in the tanks maintained randomly assigned temperature treatments of 25 °C (control), 30, 32, 33, 34, and 36 °C. There were three replicates of each treatment.

We acclimated fish to 25 °C (maximum fluctuation + 1.0 °C) for a minimum of 14 d. Once the acclimation period concluded, temperature was increased at 1 °C d⁻¹ until treatment temperatures were reached. Treatment temperatures were maintained for 30 d, which is the longest consecutive period that fish would be exposed to extreme temperatures in the San Pedro River, Arizona. The San Pedro River is currently designated as critical habitat for spikedace. In addition to standard feeding procedures, fish were fed live brine shrimp (*Artemia* spp.) to supplement diet. Approximately 20% of the tank water was removed by siphon from each tank per day and replaced with dechlorinated (*Amquel®*) municipal water to prevent the accumulation of excess food and poor water quality. Water quality in each test tank was tested once a week to monitor pH, ammonia, and dissolved oxygen levels. Water temperatures in tanks were recorded twice per day to confirm that tank temperatures remained within 1.0 °C of the test temperature. Mortalities were recorded each day and fish were preserved in 10% formalin.
Growth was averaged by tank and analyzed using multiple regression. Survival and mortality data were plotted using logistic regression for binomial counts and were used to predict the 30 d LT50, the temperature survived by 50% of the fish after 30 d. Data were analyzed with SPSS Version 12.0 and JMP Version 4.0.4 (JMP: 4.0.4 (Academic). Copyright 1989–2001).

2.3. ACE method with diurnal temperature fluctuations

Fluctuating temperatures were simulated to mimic naturally occurring conditions. Fluctuating experiments were conducted in October 2004 through December 2004 using a computerized recirculating laboratory system built specifically for this project. To control temperature within treatment tanks, we used six Hass K series Intellifaucets. Intellifaucets were remotely controlled through a National Instruments PCI 6704 installed in a desktop PC. Feedback was provided from the treatment tanks by Omega T-type thermocouples (+0.5 °C) and a National Instrument SCB-68 shielded connector block. We calibrated thermocouples against a mercury thermometer with increments of 0.1 °C. Two tanks for every set of three tanks were equipped with thermocouples. All tanks drained into a common sump tank prior to being filtered with a mechanical filter, biological filter, and UV sterilizer to prevent any differences in water quality among tanks. Filtered water was added back to the test tanks at the appropriate temperature. Approximately 1/3 of the water in each test tank was replaced every 20 min to maintain test temperatures. Thermocouples recorded tank temperatures every 20 min and an external thermometer confirmed temperature in every treatment tank twice daily. Light cycles, feeding, and cleaning methods remained the same as in the previous experiment. We monitored water quality daily throughout the experiment.

Temperature fluctuations were sinusoidal on a 24 h cycle, with the highest temperature reached at 3 pm and the lowest at 3 am. A temperature of 34 °C was chosen as the upper limit for spikedace, as it slightly exceeded the 30 d LT50 calculated from the previous experiments and was lethal within 30 d under static temperature conditions. Fluctuating temperature treatments were 30–34, 28–34 and 24–34 °C, with three replicates for each treatment. In addition, three control tanks were kept at 25 °C to control for environmental variables and 34 °C to ensure the upper temperature was lethal under static conditions.

We measured 10 randomly selected fish (20–40 mm) for each treatment tank. Fish were acclimated at 25 °C (+0.5 °C) for at least 14 d prior to starting temperature fluctuations. Temperature was increased at a rate of 1.0 °C d⁻¹ until the lowest temperature for each treatment, 24, 28, and 34 °C, was achieved. From the lowest temperature, fluctuation amplitude was increased by 1.0 °C d⁻¹ until the desired test fluctuation was achieved. Test fluctuations were maintained for 30 d and mortality recorded twice daily. Mortalities were preserved in 95% ethanol. Treatment temperatures remained within 0.5 °C of desired temperatures for the 30 d exposure. As in the previous experiment, all the fish were removed from tanks after at least 30 d exposure period and measured on the same day for growth determination. Mortality was reported for the first 30 d of the experiment.

Percent survival was calculated for each tank and all treatments compared using analysis of variance (ANOVA). Change in total length was calculated for each tank, except those in the constant 34 °C treatment, and compared using Student’s t procedure (alpha = 0.05). Data were analyzed using JMP Version 4.0.4 (JMP: 4.0.4 (Academic). Copyright 1989–2001).

2.4. Chronic lethal maximum method

Ten randomly selected fish (30–50 mm) were acclimated at 30 °C (+0.5 °C) for a 14 d period and then temperature was increased 1.0 °C d⁻¹ until all fish died. Fish remained at each temperature for 24 h. Light cycles, feeding, and cleaning methods remained the same as in the previous three experiments. Mortalities and temperatures were recorded twice daily. A two-sample t-test was used to identify differences between tanks. Data were analyzed using JMP Version 4.0.4 (JMP: 4.0.4 (Academic). Copyright 1989–2001).

3. Results

3.1. ACE method with static temperatures

We found no difference in initial mean total fish length between treatments when test tank was considered ($r^2 = 0.09, P = 0.4712$). All fish survived 14 d acclimation. All fish survived 30 d exposure to 25 °C (control) and 30 °C. No fish survived 30 d exposure to 34 or 36 °C treatments. During the 30 d exposure period the maximum time until 100% mortality occurred was 12 d at 33 °C, 7 d at 34 °C, and 2 d at 36 °C. Mortality was first observed at 34 °C during the acclimation period for the 36 °C treatment, 2 d prior to reaching the desired test temperature. Only two fish remained alive by day 1 of the 36 °C treatment. Mortality was first observed on day 10 of the 32 °C treatment and an average of 36% of fish remained alive by the end of the 30 d exposure period.

The 30 d LT50 was 32.1 °C, with a 95% confidence interval of 31.8–32.5 °C (Likelihood Ratio test, $X^2 = 29.71, P<0.0001$) (Fig. 1). The Hosmer and Lemeshow goodness-of-fit test on the regression was not significant ($X^2 = 2.758, P = 0.07$) indicating a good fit. Growth of spikedace varied significantly by treatment ($r^2 = 0.64, P<0.0001$), when controlling for treatment tank and time of year. Mean growth was inversely related to temperature (Fig. 2). Growth was highest at the 25 °C control, 16 ± 1.1 mm. The 32 °C treatment experienced the smallest amount of growth, 5.9 mm or 37% of the total growth experienced by fish in the control treatment. Growth at 30 °C was...
We observed exophthalmia, an abnormal protrusion of the eyeball from the orbit, on day 33 in fish exposed to the $30^\circ C$ treatment. Exophthalmia and red coloration around the head was observed on day 28 for fish exposed to the $32^\circ C$ treatment, as was loss of equilibrium (day 18), swimming in circles (day 24), and inactivity (day 30). The same symptoms were observed earlier in the $33^\circ C$ treatment where loss of equilibrium, exophthalmia, and inactivity were first observed on days 8 and 9.

3.2. ACE method with diurnal temperature fluctuations

No fish died during the 14 d acclimation period or temperature ramping period. No fish survived 30 d exposure to the $34^\circ C$ control treatment. All fish survived the $25^\circ C$ control treatment.

ANOVA tests for survival data indicated a significant difference among all treatments ($F = 5.80, P = 0.01$). We observed 96.5% survival for fish exposed to the control ($25^\circ C$) treatment. Similar survival rates were observed for the $24-34^\circ C$ treatment. Survival for fish exposed to the $28-34^\circ C$ (mean survival = 79.2%) and $30-34^\circ C$ fluctuation (mean survival = 73.3%) was significantly lower.

No difference in growth was observed among spikedace exposed to the three fluctuating treatments (Fig. 3). However, growth of spikedace in both the $24-34^\circ C$ and $30-34^\circ C$ was significantly lesser growth than the control treatment ($F = 4.05, P = 0.03$).

3.3. Chronic lethal maximum method

In the CLM trials, $35.6 \pm 0.4^\circ C$ was lethal for spikedace. A comparison of the two tanks indicated that test tank did not influence the upper lethal temperature (two-sample $t$-test, $P = 0.10$). All fish died prior to reaching $36^\circ C$. The lowest temperature that death was observed was $31.8^\circ C$, and the highest temperature at death was $35.9^\circ C$. Loss of equilibrium was observed at $34.5-35^\circ C$ in both tanks.

4. Discussion

The ACE with static temperatures provided the most conservative for estimating thermal tolerance. The slower rate of change used during the CLM trials allowed fish to acclimate to changing temperature and simultaneously allowed more time for temperature to act as a lethal agent (Beitinger et al., 2000). The LT50, calculated using the ACE method with static temperatures, was $32.1^\circ C$. Fish exhibited obvious signs of stress at test temperatures below the LT50 indicating that there are extremely high-energy costs associated with remaining at temperatures close to the upper thermal limit for a long period of time (Lowe and
Fish subjected to high temperature experience high metabolic demands that can result in sub-lethal effects such as disease and suppressed growth (Brett, 1956; Dickerson and Vinyard, 1999; Johnstone and Rahel, 2003). Exophthalmia was observed in fish exposed to all treatments above 30°C. Exophthalmia is commonly observed in fish that are stressed and is often cited as a symptom for a variety of diseases. Loss of equilibrium, swimming in circles, and inactivity were observed at temperatures beyond 32°C. We observed reduced growth at temperatures as low as 30°C. Spikedace typically experience rapid growth in their first summer and autumn obtaining lengths of 35–40 mm (Probst et al., 1986). Growth typically does not occur in winter. Suppressed growth at high temperature may have serious consequences for the survival of spikedace. The fecundity of a female spikedace is directly related to the amount of growth obtained in her first year of life, as spikedace only live for 1–2 yr in the wild (Barber et al., 1970).

The calculation of an LT50 is a common method for determining the tolerance limit of a species. However, as in our study, tests often are conducted in the absence of naturally occurring environmental stressors. In the presence of additional stressors, such as predation, interspecific competition, disease and foraging, a lower temperature may be lethal to spikedace as synergy between temperature and other stressors can occur (Relyea, 2004). Decreased oxygen availability at high temperatures combined with an elevated oxygen demand due to increased metabolic rate create conditions where fish cannot consume enough oxygen to survive. High temperatures further accelerate this process by altering hemoglobin to the point that a fish can no longer pick up oxygen at its gills, resulting in suffocation (Helfman et al., 1999). The fish we used were provided ample amounts of food and oxygen, treated for disease, and free of predators. These are all factors that may impact survival in the wild. Our data suggest that a more conservative estimate of upper thermal tolerance for spikedace may be 30°C, as no mortality was observed at this temperature.

A natural diel cycling of temperature did extend the upper thermal tolerance of spikedace. Spikedace were able to survive brief exposure to 34°C, which was lethal under static conditions. Under a natural diurnal cycle, brief exposure to high temperature would likely be less stressful for fish, as low temperatures provide temporal refuge (Castleberry and Cech, 1986). Natural diel cycles can increase tolerance to high temperature (Hubbs, 1964; Selong et al., 2001; Johnstone and Rahel, 2003) as fish often acclimate to the highest temperature to which they are exposed (Heath, 1963). Such adaptations may allow some species to briefly survive exposure to temperatures beyond their thermal limits. Fish that evolved with large diel and annual fluctuations in temperature are commonly equipped with cellular and sub-cellular mechanisms for coping with temperature changes. Acute heat stress initiates the production of heat shock proteins, which reconfigure proteins that become denatured at high temperatures (Helfman et al., 1999). Desert pupfish Cyprinodon macularius can live at temperatures just below their lethal limit (Lowe and Heath, 1969). At such high temperature, metabolic demands are so great that pupfish are required to seek food continuously. Examination of stomach contents indicates that food assimilation takes place later in the day, when temperatures are lower. A similar pattern was noted in Lahontan cutthroat trout Oncorynchus clarki henshawi, which stopped eating at high temperature but quickly recovered their appetite when temperatures were lowered (Dickerson and Vinyard, 1999). Fish may also find relief from high temperatures within cooler microhabitats found throughout a stream. Temperature differences within a small Arizona stream can exceed 12°C among habitat types (Deacon and Minklely, 1974). Temperature orientation and selection are behaviors exhibited by most freshwater fish species (Norris, 1963). Longfin dace Agosis chrysoaster may avoid high temperatures within the stream channel by congregating in shaded pools where temperatures are 3°C lower than the main channel (Deacon and Minklely, 1974). Although temperatures above the thermal limit of spikedace are frequently recorded within their habitat, different behaviors and cellular mechanisms may allow them to persist in the wild.

Data collected in other studies indicate that many nonnative species have a higher thermal tolerance than the spikedace. Using the CTM, Carveth et al. (2006) determine that red shiner, green sunfish and yellow bullhead all have a significantly higher thermal tolerance than spikedace. Red Shiner have been identified as the most serious threat to the remaining spikedace populations through competitive displacement (Douglas et al., 1994). A higher thermal tolerance may provide nonnative species such as the red shiner with a competitive advantage at high temperatures. For example, a higher thermal tolerance was cited as a reason for the success of the introduced Arroyo chub Gila orcutti in displacing native Mojave tui chub Gila bicolor mohavensis in the Mohave River (Castleberry and Cech, 1986). Species with a higher thermal tolerance are able to use a larger portion of the stream, providing them with more foraging opportunities and areas to avoid predators.

Measurements of upper lethal tolerance may be unrealistic as fish rarely experience such high temperatures in the wild (Brett, 1956; Lutterschnidt and Hutchison, 1997). However, temperatures in small Arizona streams reach daily maxima of 24–40.3°C (Deacon and Minklely, 1974; US Geologial Survey, 2005), and mortality related to high temperature is recorded frequently. Significant alterations have occurred in Arizona’s streams and consequently increasing stream temperatures have been recorded since the early 1900s. Long-term exposure to temperatures commonly encountered in Arizona streams proved to be lethal for spikedace. Data from the San Pedro River, Arizona were used to determine the treatment temperatures
and the length of exposure time used in this study. Peaks above 30°C are commonly observed in the San Pedro River for periods of 30 d.

In summary, the chronic temperature experiments indicate that temperatures within spikedace habitat should remain below 30°C to reduce stress and mortality. Although spikedace are able to survive brief exposure to temperatures above their upper lethal limit, there is a cost, in this case reduced growth, associated with survival. Stream temperature fluctuations above 30°C may reduce the available habitat by forcing fish into areas of thermal refuge. Spikedace have a relatively low upper thermal tolerance when compared to other nonnative species. Stress from interspecific competition, specifically with the red shiner, may further reduce the thermal tolerance of spikedace. A more detailed examination of spikedace survival and predation by nonnative species. Although this study focuses solely on the upper thermal tolerance of spikedace, there is a need to also examine the lower lethal tolerance and physiological optimum of this species to determine how natural daily and annual temperature fluctuations affect the growth and survival of spikedace.

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